Metabolism of Nitrilotriacetic Acid (NTA) in the Mouse

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INTRODUCTION

Nitrilotriacetic acid (NTA) is a good sequestering agent and is being used as a partial replacement for the phosphates in household detergent formulations. Despite the fact that the metabolism of this compound has been studied in the rat, rabbit, dog, monkey (MICHAEL and WAKIM, 1971) and in humans (BUNDY and ARNOLD, 1973) pharmacokinetic data for NTA in the mouse have been lacking. A previous report dealt with distribution of ^{14}C-NTA in mice by whole-body autoradiography (TJÄLVE, 1972) but no absorption and metabolic excretion data were available. The present studies were carried out as part of a general program on NTA and were designed to determine the absorption, distribution and metabolic excretion of NTA in the mouse.

METHODS

Nitrilotriacetic acid (carboxyl ^{14}C, specific activity 58 mCi/mmol) was purchased from Amersham Searle. Radiochemical purity was found by thin-layer chromatography (TLC) to be greater than 98%. This compound was diluted with nonradioactive NTA (Aldrich Chemical) to 0.222 µCi/mg and was used in all experiments.

Five male albino mice (BioBreeding Laboratory, Ottawa) weighing 20-30 g were given a single oral dose of ^{14}C-NTA (180 mg/kg, 40 µCi) in phosphate buffer (pH 7.4, 2 ml/kg). Serial blood samples (10 µl) were taken from the tail at 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hr after administration for the analysis of radioactivity. The animals were exsanguinated after the last sampling and the liver, brain, heart, kidney, lung, spleen, bladder, testes, a portion of the femur, muscle, skin and fat were excised for the analysis of radioactivity. Serial blood samples (10 µl) were also collected from five tail-vein dosed mice (45 mg/kg, 10 µCi) at 2 min intervals for 20 min then at 30 min and 60 min after administration. The tissues were similarly excised following exsanguination for the estimation of radioactivity.

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Two groups of twenty mice were dosed with ^{14}C-NTA, 180 mg/kg orally and 45 mg/kg intravenously respectively. Five animals from each group were exsanguinated at 1, 8, 24 and 48 hr after administration, and the tissues were excised for estimation of radioactivity.

For excretion studies five mice were given a single oral dose of ^{14}C-NTA (180 mg/kg, 40 µCi) and were individually housed in the metabolic cages with food and water ad libitum. Urine and fecal samples were collected daily for seven days.

The bile ducts of four mice were cannulated with heat stretched PE-10 polyethylene tubing. The body temperature of the animals were monitored with a rectal thermistor and maintained between 36.2 - 37.2°. These mice were divided into two groups and were dosed I.V. with ^{14}C-NTA (45 mg/kg and 4.5 mg/kg) in the phosphate buffer via the tail vein. Bile samples were collected hourly for 8 hr for the assessment of radioactivity. All tails of the I.V. dosed mice were analyzed for radioactivity at the end of experiments and any animal possessing more than 5% of the dose in the tail were rejected from the experiment.

Sample Preparations:

Blood: The blood samples (10 µl) were dissolved in a mixture of Soluene 350: 2-propanol (1 ml, 1:1, Packard) and decolorized with 30% hydrogen peroxide (0.5 ml) followed by incubation at 50° for 30 min. After cooling the mixture was dissolved in Dimulune-30 (Packard) and counted for radioactivity using a Mark III Liquid Scintillation System (Amersham Searle). Quenching was corrected for with an external standard.

Tissues: The tissue sample (0.01 0.1 g) was digested with Soluene-3 50 (1 ml) for 12 hr and the digest was dissolved in Dimulune-30 (15 ml) for the assessment of radioactivity. The femur was combusted in a Tri Carb Oxidizer (Packard); the $^{14}CO_2$ was trapped in ethanolamine and counted in Dimulune-30.

Urine and bile: The urine and bile samples (100 µ l) were dissolved in Aquasol (15 ml, New England Nuclear) and were counted directly.

Feces: Feces were extracted with ethanol (30 ml) and the ethanol extract (1 ml) was dissolved in Dimulune-30 (15 ml) for the assessment of radioactivity.

Urine was streaked on a silica gel plate (1000 µ, Analtech) and developed with ascending n-butanol-pyridine-water (1:1:1). The band containing radioactive

material as detected by an Actigraph III (Nuclear Chicago) was removed from the plate and extracted with ethanol (50 ml). The concentrated extract was methylated with diazomethane (Diazald, Aldrich) and examined by a gas chromatograph-mass spectrometer (GC-MS) at 70 ev (Finnigan 4000 GC-MS System) equipped with a 6' X 1/8" id glass column packed with 3% OV-17 on 80-100 mesh Chromosorb WHP and a flame ionization detector. The radioactive material in the ethanolic extract was determined in a manner similar to that described for the urine.

RESULTS

Determination of radioactivity in the blood samples of rats dosed orally with ^{14}C-NTA showed that the peak level occurred at 1 hr after administration. The blood concentration rapidly declined to 1/2 and 1/5 of the initial highest level at 2 hr and 3 hr after dosing, respectively. At 4 hr ca. 85% of the radioactivity was removed from the blood (Table 1).

TABLE 1

Blood concentrations of Radioactivity after (A) Oral Administration of ^{14}C-Nitrilotriacetic Acid (180 mg/kg 40 µC) and (B) I.V. Dose (45 mg/kg 10 µCi). Results are Expressed as Mean ± SD (dpm/µl Blood) For Five Mice.

Time (hr)	Concentration (dpm/µl)	Time (min)	Concentration (dpm/µl)
0.5	278 ± 150	2	563 ± 24
1	299 ± 89	4	564 ± 63
2	150 ± 98	6	509 ± 81
3	56 ± 24	8	507 ± 103
4	43 ± 9	10	430 ± 58
5	37 ± 5	12	433 ± 115
6	34 ± 3	14	400 ± 107
7	37 ± 18	16	362 ± 91
8	40 ± 15	18	400 ± 15
		20	347 ± 57
		30	242 ± 104
		60	99 ± 35

Blood concentrations of radioactivity were also estimated in the mice dosed intravenously with ^{14}C-NTA. The blood concentrations were 3/5, 2/5 and 1/5 of the initial highest concentration at 20 min, 30 min and 60 min after administration, respectively. Since all animals died immediately after I.V. dosing at 180 mg/kg the dose level was reduced to 45 mg/kg. All tissues were assessed for radioactivity with an appreciable amount being detected in the 1 hr tissues (Table 2) but not in the 8, 24 and 48 hr tissues.

TABLE 2

Tissue Distribution of Radioactivity at 1 hr After (A) Oral Dose of ^{14}C-Nitrilotriacetic Acid (180 mg/kg 40 µCi). (B) I.V. Administration 45 mg/kg 10 µCi). Results are Given in Mean ± SD (dpm/mg Tissue) for Five Mice.

	A Concentration (dpm/mg)	B Concentration (dpm/mg)
Liver	1.46 ± 0.38	1.84 ± 0.30
Kidney	5.73 ± 0.47	19.4 ± 2.5
Spleen	0.83 ± 0.24	0.86 ± 0.19
Brain	0.36 ± 0.10	0.25 ± 0.07
Lung	2.15 ± 0.62	2.6 ± 0.50
Intestine	2.33 ± 0.34	2.2 ± 0.43
Bladder	82.4 ± 2.7	109. ± 45.
Heart	0.36 ± 0.07	0.86 ± 0.14
Skin	0.86 ± 0.38	2.5 ± 0.62
Muscle	1.34 ± 0.78	2.9 ± 2.6
Fat	0.9 ± 0.07	1.6 ± 0.34
Testes	1.20 ± 0.23	0.40 ± 0.03
Bone	33.5 ± 7.5	28.0 ± 4.1

The kidney, bladder and bone of both oral and I.V. dosed mice were shown to possess higher concentrations than other tissues. The route of administration appeared to affect the pattern of distribution as higher concentrations of radioactivity were found in the kidney, heart and skin of the I.V. dosed rats. Excretion of ^{14}C-NTA following a single oral dose was studied. Approximately 99% of the dose was eliminated within 24 hr. Of this, 96 ± 6.4% (mean ± SD) was removed from the urine and 3.5 ±

1.3 (mean ± SD) in the feces. Examination of the bile indicated that little radioactivity was present (< 1%).

There was only one radioactive band found in the TLC plate of the urine and feces. The Rf value (0.54) was found to be identical to that of the authentic ^{14}C-NTA chromatographed alongside the extracts. GC-MS analysis of the methylated extracts showed a weak but significant molecular ion at m/e 233 ($C_9H_{15}NO_6$)$^+$, characteristic of the trimethyl ester of NTA.

DISCUSSION

The fact that the peak blood concentration occurred at 1 hr after administration demonstrated that NTA was readily absorbed from the gastro-intestinal tract of mice. MICHAEL and WAKIM (1971) noted that NTA was well absorbed in fasted rats and dog but not in the rabbit and monkey. Our experiments were carried out on non-fasted animals to preclude stressful condition of food deprivation. Since NTA is a tricarboxylic acid it would be expected to be rapidly removed from the blood. This was found to be the case. NTA was rapidly distributed into all tissues with the bladder, kidney and bone possessing higher concentrations. This result is consistent with that of TJALVE (1972). It is conceivable that the bladder and kidney would show higher content than other tissues since these organs were the primary route for the excretion of NTA. Accumulation of NTA in the bone of the rat, rabbit, dog and monkey has been reported (MICHAEL and WAKIM 1971). It is thus not surprising that NTA is selectively accumulated in the bones of the mouse. Elimination of NTA from the skeletal tissue was also rapid, as was indicated by the lack of any detectable amount of radioactivity in this tissue at 8 hr after administration. This result implies that it is not likely that NTA would cause any serious adverse effect to the bone of mice because of its short half-life in this tissue.

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